Inventor search

Helms 09/250,056

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=> fil medline biosis wpids hcaplus
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     FILE 'MEDLINE, BIOSIS, WPIDS, HCAPLUS' ENTERED AT 14:44:24 ON 14 OCT 1999
                E MARKS J/AU
L1           794 S E3 OR E8-9
                E MARKS JAMES/AU
L2            75 S E3-5
                E POUL M/AU
L3            45 S E3-4 OR E6
L4           906 S L1 OR L2 OR L3
L5          8651 S ERBB2 OR ERBB 2 OR ERB B2
L6            43 S L4 AND L5
L7        928909 S INTERNAL? OR CHIMER? OR FUSION?
L8            35 S L6 AND ANTIBOD?
L9             8 S L6 AND L7
L10           36 S L8 OR L9
L11           18 DUP REM L10 (18 DUPLICATES REMOVED)

     FILE 'MEDLINE, BIOSIS, WPIDS, HCAPLUS' ENTERED AT 14:46:58 ON 14 OCT 1999

=> d bib ab 1-18

L11  ANSWER 1 OF 18  HCAPLUS  COPYRIGHT 1999 ACS
AN   1999:63504  HCAPLUS
DN   130:152229
TI   Efficient construction of a large nonimmune phage antibody
     library: the production of high-affinity human single-chain
     antibodies to protein antigens. [Erratum to document cited in
     CA129:107708]
AU   Sheets, Michael D.; Amersdorfer, Peter; Finnern, Ricarda; Sargent, Peter;
     Lindquist, Ericka; Schier, Robert; Hemingsen, Grete; Wong, Cindy;
Gerhart,
     John C.; Marks, James D.
CS   Department of Mol. Cell Biol., Univ. California, Berkeley, CA, 94720, USA
SO   Proc. Natl. Acad. Sci. U. S. A. (1999), 96(2), 795
     CODEN: PNASA6; ISSN: 0027-8424
PB   National Academy of Sciences
DT   Journal
LA   English
AB   The fifth author's name was spelled incorrectly; the correct spelling is
     Ericka Lindquist.  Her affiliation should be "Program in Infectious
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Diseases, School of Public Helath, University of California, Berkeley, CA 94720.".

L11 ANSWER 2 OF 18 MEDLINE
AN 1999376336 MEDLINE
DN 99376336
TI Isolation and characterization of an anti-CD16 single-chain Fv fragment and construction of an anti-HER2/neu/anti-CD16 bispecific scFv that triggers CD16-dependent tumor cytolysis.
AU McCall A M; Adams G P; Amoroso A R; Nielsen U B; Zhang L; Horak E; Simmons H; Schier R; **Marks J D**; Weiner L M
CS Fox Chase Cancer Center, Philadelphia, PA 19111, USA.
NC CA65559 (NCI)
CA06927 (NCI)
CA50633 (NCI)
SO MOLECULAR IMMUNOLOGY, (1999 May) 36 (7) 433-45.
Journal code: NG1. ISSN: 0161-5890.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199910
EW 19991004
AB Bispecific **antibody** (bsAb)-based clinical trials of cancer have been conducted primarily using intact murine monoclonal **antibody** (mAb)-derived molecules. In some of these trials, toxicity resulting from the interactions of **antibody** Fc domains with cellular Fc receptors has limited the doses of **antibody** (Ab) that can be employed. Furthermore, human anti-mouse Ab responses prohibit multiple therapy courses. These factors have decreased the efficacy of the bsAb 2B1, which targets the extracellular domains (ECD) of the HER2/neu protooncogene product and the human FcgammaRIII (CD16). To address these obstacles, we have constructed and characterized a fully human gene-fused bsAb from single-chain Fv (scFv) molecules specific for HER2/neu and CD16.
The human anti-CD16 scFv component, NM3E2, was isolated from a human scFv phage display library. As binding of NM3E2 to human neutrophil-associated CD16 decreased in the presence of plasma IgG, we have concluded that NM3E2 recognizes an epitope in the vicinity of the Fc binding pocket. Furthermore, the NM3E2 scFv was found by surface plasmon resonance-based epitope mapping to share an overlapping epitope with the Leu-11c mAb. The human anti-HER2/neu scFv component, C6.5, which was previously isolated from a human scFv phage display library, was employed as **fusion** partner for the creation of a bispecific scFv (bs-scFv). In the presence of the C6.5 x NM3E2 bs-scFv, peripheral blood lymphocytes promoted significant lysis of human SK-OV-3 ovarian cancer cells overexpressing HER2/neu. Biodistribution studies performed in SK-OV-3 tumor-bearing scid mice revealed that 1% ID/g of 125I-labeled C6.5 x NM3E2 bs-scFv was specifically retained in tumor at 23 h following injection. These results indicated that both scFv components of the bs-scFv retained their function in the **fusion** protein. This bsAb should overcome some of the problems associated with the 2B1 bsAb. C6.5 x NM3E2 bs-scFv offers promise as a platform for multifunctional binding proteins with potential clinical

applications as a result of its human origin, lack of an Fc domain, ease of production, high level of in vitro tumor cell cytotoxicity and highly selective tumor targeting.

L11 ANSWER 3 OF 18 MEDLINE DUPLICATE 1
AN 1999160873 MEDLINE
DN 99160873
TI Toward selection of **internalizing antibodies** from phage libraries.
AU Becerril B; **Poul M A; Marks J D**
CS Department of Pharmaceutical Chemistry, University of California, San Francisco 94110, USA.
SO BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1999 Feb 16) 255 (2)
386-93.
Journal code: 9Y8. ISSN: 0006-291X.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199905
EW 19990502
AB **Antibodies** which bind cell surface receptors in a manner whereby they are endocytosed are useful molecules for the delivery of drugs, toxins, or DNA into the cytosol of mammalian cells for therapeutic applications. Traditionally, **internalizing antibodies** have been identified by screening hybridomas. For this work, we studied a human scFv (C6.5) which binds **ErbB2** to determine the feasibility of directly selecting **internalizing antibodies** from phage libraries and to identify the most efficient display format. Using wild-type C6.5 scFv displayed monovalently on a phagemid, we demonstrate that anti-**ErbB2** phage **antibodies** can undergo receptor-mediated endocytosis. Using affinity mutants and dimeric diabodies of C6.5 displayed as either single copies on a phagemid or multiple copies on phage, we define the role of affinity, valency, and display format on phage endocytosis and identify the factors that lead to the greatest enrichment for **internalization**. Phage displaying bivalent diabodies or multiple copies of scFv were more efficiently endocytosed than phage displaying monomeric scFv and recovery of infectious phage was increased by preincubation of cells with chloroquine.
Measurement of phage recovery from within the cytosol as a function of applied phage titer indicates that it is possible to select for endocytosable **antibodies**, even at the low concentrations that would exist for a single phage **antibody** member in a library of 10(9). Copyright 1999 Academic Press.

L11 ANSWER 4 OF 18 MEDLINE
AN 1999101498 MEDLINE
DN 99101498
TI Redirecting effector T cells through their IL-2 receptors.
AU Lustgarten J; **Marks J**; Sherman L A
CS Department of Immunology, The Scripps Research Institute, La Jolla, CA 92037, USA.
NC CA57855 (NCI)
CA25803 (NCI)
SO JOURNAL OF IMMUNOLOGY, (1999 Jan 1) 162 (1) 359-65.

Journal code: IFB. ISSN: 0022-1767.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals; Cancer Journals
EM 199903
EW 19990304
AB **Fusion** proteins constructed of a tumor-specific Ab joined to IL-2 (Ab-IL-2) have been used in the past to deliver cytokine directly to the site of tumor cells in vivo. These molecules mimic the activity of IL-2 and assist in activating and expanding antitumor effector cells. To enhance the cytolytic activity of CTL specific for peptide epitopes of the Her-2/neu tumor Ag presented by HLA-A*0201 molecules, a **fusion** protein was constructed consisting of a single chain Ab specific for Her-2/neu, linked to IL-2 (neu-Ab-IL-2). When added to a mixture of tumor cells and Her-2/neu-specific CTL, the protein was found to augment lysis of tumor cells. In addition, the hybrid molecule also promoted lysis of Her-2/neu expressing tumors by non-tumor-specific cloned T cell lines, including Th1 CD4 cells. Analysis of the mechanism of cytotoxicity revealed that the **fusion** protein mediates the formation of stable conjugates between T cells expressing IL-2R and tumor cells expressing Her-2/neu, resulting in lysis through the Fas-Fas ligand pathway. Lysis induction was independent of specific engagement by the TCR. When tested for its ability to enhance tumor cell eradication by Her-2/neu-specific CD8+ T cells in an adoptive transfer model in SCID mice, neu-Ab-IL-2 facilitated the elimination of tumor cells in vivo. Surprisingly, the combination of non-tumor-specific CD8+ T cells and **fusion** protein also induced a significant delay of tumor growth. This represents a novel approach for redirecting non-tumor-specific T cells to eliminate tumors.

L11 ANSWER 5 OF 18 MEDLINE DUPLICATE 2
AN 1999263530 MEDLINE
DN 99263530
TI Targeted gene delivery to mammalian cells by filamentous bacteriophage.
AU **Poul M A; Marks J D**
CS Department of Anesthesia and Pharmaceutical Chemistry, University of California, San Francisco General Hospital, 1001 Potrero Avenue, San Francisco, CA 94110, USA.
SO JOURNAL OF MOLECULAR BIOLOGY, (1999 Apr 30) 288 (2) 203-11.
Journal code: J6V. ISSN: 0022-2836.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199908
EW 19990801
AB We report that prokaryotic viruses can be re-engineered to infect eukaryotic cells resulting in expression of a reporter gene inserted into the bacteriophage genome. Phage capable of binding mammalian cells expressing the growth factor receptor **ErbB2** and undergoing receptor-mediated endocytosis were isolated by selection of a phage **antibody** library on breast tumor cells and recovery of infectious phage from within the cell. As determined by immunofluorescence, F5 phage were efficiently endocytosed into 100 % of **ErbB2** expressing SKBR3 cells. To achieve reporter gene expression, F5 phage were engineered

to package the green fluorescent protein (GFP) reporter gene driven by the CMV promoter. These phage when applied to cells underwent **ErbB2**-mediated endocytosis leading to GFP expression. GFP expression occurred only in cells overexpressing **ErbB2**, was dose-dependent reaching, 4 % of cells after 60 hours and was detected with phage titers as low as 2.0 x 10(7) cfu/ml (500 phage/cell). The results demonstrate that bacterial viruses displaying the appropriate **antibody** can bind to mammalian receptors and utilize the endocytic pathway to infect eukaryotic cells, resulting in expression of a reporter gene inserted into the viral genome. This represents a novel method to discover targeting molecules capable of delivering a gene intracellularly into the correct trafficking pathway for gene expression by directly screening phage **antibodies**. This should significantly facilitate the identification of appropriate targets and targeting molecules for gene therapy or other applications where delivery into the cytosol is required.

This approach can be adapted to directly select, rather than screen, phage **antibodies** for targeted gene expression. The results also demonstrate the potential of phage **antibodies** as an in vitro or in vivo targeted gene delivery vehicle. Copyright 1998 Academic Press.

L11 ANSWER 6 OF 18 MEDLINE DUPLICATE 3
AN 1998263325 MEDLINE
DN 98263325
TI Efficient construction of a large nonimmune phage **antibody** library: the production of high-affinity human single-chain **antibodies** to protein antigens [published erratum appears in Proc Natl Acad Sci U S A 1999 Jan 19;96(2):795].
AU Sheets M D; Amersdorfer P; Finnern R; Sargent P; Lindquist E; Schier R; Hemingsen G; Wong C; Gerhart J C; **Marks J D**; Lindqvist E/SS/[corrected to Lindquist E]
CS Department of Molecular Cell Biology, University of California, Berkeley, CA 94720, USA.
NC GM15203-02 (NIGMS)
R01 GM19363 (NIGMS)
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1998 May 26) 95 (11) 6157-62.
Journal code: PV3. ISSN: 0027-8424.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Cancer Journals; Priority Journals
EM 199808
AB A large library of phage-displayed human single-chain Fv **antibodies** (scFv), containing 6.7 x 10(9) members, was generated by improving the steps of library construction. Fourteen different protein antigens were used to affinity select **antibodies** from this library. A panel of specific **antibodies** was isolated with each antigen, and each panel contained an average of 8.7 different scFv. Measurements of **antibody**-antigen interactions revealed several affinities below 1 nM, comparable to affinities observed during the secondary murine immune response. In particular, four different scFv recognizing the **ErbB2** protein had affinities ranging from 220 pM

to 4 nM. **Antibodies** derived from the library proved to be useful reagents for immunoassays. For example, **antibodies** generated to the Chlamydia trachomatis elementary bodies stained Chlamydia-infected cells, but not uninfected cells. These results demonstrate that phage **antibody** libraries are ideally suited for the rapid production of panels of high-affinity mAbs to a wide variety of protein antigens. Such libraries should prove especially useful for generating reagents to study the function of gene products identified by genome projects.

L11 ANSWER 7 OF 18 MEDLINE DUPLICATE 4
AN 1998314863 MEDLINE
DN 98314863
TI Prolonged in vivo tumour retention of a human diabody targeting the extracellular domain of human HER2/neu.
AU Adams G P; Schier R; McCall A M; Crawford R S; Wolf E J; Weiner L M; **Marks J D**
CS Department of Medical Oncology, Fox Chase Cancer Center, Philadelphia, PA 19111, USA.
NC CA 65559 (NCI)
CA06927 (NCI)
SO BRITISH JOURNAL OF CANCER, (1998 May) 77 (9) 1405-12.
Journal code: AV4. ISSN: 0007-0920.
CY SCOTLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199809
EW 19980902
AB Single-chain Fv (scFv) molecules exhibit highly specific tumour-targeting properties in tumour-bearing mice. However, because of their smaller size and monovalent binding, the quantities of radiolabelled scFv retained in tumours limit their therapeutic applications. Diabodies are dimeric **antibody**-based molecules composed of two non-covalently associated scFv that bind to antigen in a divalent manner. In vitro, diabodies produced from the anti-HER2/neu (c-**erbB-2**) scFv C6.5 displayed approximately 40-fold greater affinity for HER2/neu by surface plasmon resonance biosensor measurements and significantly prolonged association with antigen on the surface of SK-OV-3 cells (t1/2 cell surface retention of > 5 h vs 5 min) compared with C6.5 scFv. In SK-OV-3 tumour-bearing scid mice, radioiodinated C6.5 diabody displayed a highly favourable balance of quantitative tumour retention and specificity. By as early as 4 h after i.v. administration, significantly more diabody was retained in tumour (10 %ID g(-1)) than in blood (6.7 %ID ml(-1)) or normal tissue (liver, 2.8 %ID g(-1); lung, 7.1 %ID g(-1); kidney, 5.2 %ID g(-1)).
Over the next 20 h, the quantity present in blood and most tissues dropped approximately tenfold, while the tumour retained 6.5 %ID g(-1) or about two-thirds of its 4-h value. In contrast, the 24-h tumour retention of radioiodinated C6.5 scFv monomer was only 1 %ID g(-1). When diabody retentions were examined over the course of a 72-h study and cumulative area under the curve (AUC) values were determined, the resulting tumor-organ AUC ratios were found to be superior to those previously reported for other monovalent or divalent scFv molecules. In conclusion, the diabody format provides the C6.5 molecule with a distinct in vitro and

in vivo targeting advantage and has promise as a delivery vehicle for therapeutic agents.

L11 ANSWER 8 OF 18 MEDLINE DUPLICATE 5
AN 1998117298 MEDLINE
DN 98117298
TI Increased affinity leads to improved selective tumor delivery of single-chain Fv **antibodies**.
AU Adams G P; Schier R; Marshall K; Wolf E J; McCall A M; **Marks J D** ; Weiner L M
CS Department of Medical Oncology, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111, USA.. gadams@fccc.edu
NC CA 65559 (NCI)
CA06927 (NCI)
U01CA51880 (NCI)
SO CANCER RESEARCH, (1998 Feb 1) 58 (3) 485-90.
Journal code: CNF. ISSN: 0008-5472.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199804
EW 19980404
AB Mr 25,000 single-chain Fv (scFv) molecules are rapidly eliminated from the circulation of immunodeficient mice, yielding highly specific retention of small quantities of scFv in human tumor xenografts. We postulated that the specific retention of scFv in tumor could be enhanced by engineering significant increases in the affinity of the scFv for its target antigens. Affinity mutants of the human anti-HER2/neu (c-**erbB-2**) scFv C6.5 were generated by site-directed mutagenesis, which target the same antigenic epitope with a 320-fold range in affinity (3.2 x 10(-7) to 1.0 x 10(-9) M). In vitro, the Kd of each scFv correlated closely with the duration of its retention on the surface of human ovarian carcinoma SK-OV-3 cells overexpressing HER2/neu. In biodistribution studies performed in scid mice bearing established SK-OV-3 tumors, the degree and specificity of tumor localization increased significantly with increasing affinity. At 24 h after injection, tumor retention of the highest affinity scFv was 7-fold greater than that of a mutant with 320-fold lower affinity for HER2/neu. Because the rapid renal clearance of scFv may blunt the impact of improved affinity on tumor targeting, the distributions were also assayed in the absence of renal clearance (e.g., in mice rendered surgically anephric). In this model, the peak tumor retentions of the two higher affinity scFv approximated that reported previously for IgG targeting the same SK-OV-3 tumors in scid mice with intact kidneys. In contrast, the mutant with the lowest affinity for HER2/neu failed to accumulate in tumor, indicating the presence of an affinity threshold that must be exceeded for active in vivo tumor uptake. These results indicate that affinity can significantly impact the in vivo tumor-specific retention of scFv molecules.

L11 ANSWER 9 OF 18 HCAPLUS COPYRIGHT 1999 ACS
AN 1999:19920 HCAPLUS
DN 130:200834
TI Anti-HER2 immunoliposomes for targeted drug delivery
AU Park, J. W.; Kirpotin, D.; Hong, K.; Colbern, G.; Shalaby, R.; Shao, Y.; Meyer, O.; Nielsen, U.; **Marks, J.**; Benz, C. C.; Papahadjopoulos, D.
CS Division of Hematology/Oncology, University of California, San Francisco, San Francisco, CA, 94143, USA
SO Med. Chem. Res. (1998), 8(7/8), 383-391
CODEN: MCREEB; ISSN: 1054-2523
PB Birkhaeuser Boston
DT Journal
LA English
AB Anti-HER2 immunoliposomes (ILs) combine the tumor-targeting properties of certain anti-HER2 monoclonal antibodies (MAb) with the pharmacokinetic and drug delivery properties of sterically stabilized liposomes (Ls). Anti-HER2 ILs efficiently bind to and internalize in HER2-overexpressing cells in vitro, resulting in intracellular drug delivery. Localization studies in tumor xenograft models confirm that anti-HER2 ILs, unlike liposomes, internalize in tumor cells in vivo. Gold-loaded ILs accumulate intracellularly in the cytoplasm of tumor cells, while liposomes lacking MAb targeting accumulate extracellularly or within macrophages. This novel mechanism of targeted, intracellular delivery may account for the significantly enhanced antitumor efficacy of anti-HER2 ILs in vivo. Therapy studies demonstrate that delivery of doxorubicin (dox) via anti-HER2 ILs-dox greatly increases the therapeutic index of dox, both by increasing antitumor efficacy and by reducing systemic toxicity. Anti-HER2 ILs-dox produce marked therapeutic results in multiple HER2-overexpressing tumor xenograft models, including growth inhibition, regressions, and cures. Anti-HER2 ILs-dox is significantly superior to all other relevant treatment conditions, including free dox, liposomal dox, free MAb, and combinations. In addn. to dox, anti-HER2 ILs can in principle be constructed for tumor-targeted delivery of a wide variety of anticancer agents, including alternate small mol. chemotherapeutics, antisense oligonucleotides, and therapeutic genes.

L11 ANSWER 10 OF 18 WPIDS COPYRIGHT 1999 DERWENT INFORMATION LTD
DUPLICATE 6
AN 1997-077488 [07] WPIDS
DNN N1997-064299 DNC C1997-024928
TI New C6 human **antibody** binding specifically to c-**erbB-2** - useful for treatment and diagnosis of tumours, with reduced risk of generating immune response.
DC B04 D16 S03
IN **MARKS, J D**; SCHIER, R
PA (REGC) UNIV CALIFORNIA
CYC 22
PI WO 9700271 A1 19970103 (199707)* EN 117p
RW: AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE
W: AU CA JP MX
AU 9661133 A 19970115 (199718)
EP 873363 A1 19981028 (199847) EN

R: AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
ADT WO 9700271 A1 WO 1996-US10287 19960613; AU 9661133 A AU 1996-61133 19960613; EP 873363 A1 EP 1996-918487 19960613, WO 1996-US10287 19960613
FDT AU 9661133 A Based on WO 9700271; EP 873363 A1 Based on WO 9700271
PRAI US 1995-250 19950615; US 1995-238 19950614
AB WO 9700271 A UPAB: 19970212
A C6 human **antibody** (Ab) that binds specifically to c-**erbB-2** is new. Also new are: (1) nucleic acid (I) encoding Ab; (2) cell contg. recombinant (I); (3) **chimeric** molecule (II) that binds specifically to tumour cells carrying c-**erbB-2**, consisting of an effector cpd. attached to the Ab; (4) polypeptide (A) contg. 1 or more complementarity determining regions (CDR) having amino acid sequences tabulated in the specification; (5) nucleic acid (Ia) encoding a single chain polypeptide (B) with the binding specificity of Ab and comprising the binding portions of variable regions of light and heavy chains of Ab, joined by a linker; (6) (B); and (7) expression cassettes contg. (Ia) and control sequences.

USE - Where the effector cpd. is a cytotoxin, (II) are used to inhibit growth of c-**erbB-2** positive tumours (esp. breast and other carcinomas) and where it is a label they are used to detect such cells, including in vivo localisation (claimed). Ab can also be used for diagnosis/localisation, in vivo or in vitro, esp. by immunoassay. (I) and (Ia) are used to produce recombinant proteins by standard methods (claimed).

ADVANTAGE - Unlike known anti-c-**erbB-2** **antibodies**, Ab are fully human, so should elicit little, if any, immunogenic response.
Dwg.0/4

L11 ANSWER 11 OF 18 MEDLINE DUPLICATE 7
AN 97078813 MEDLINE
DN 97078813
TI Isolation of picomolar affinity anti-c-**erbB-2** single-chain Fv by molecular evolution of the complementarity determining regions in the center of the **antibody** binding site.
AU Schier R; McCall A; Adams G P; Marshall K W; Merritt H; Yim M; Crawford R S; Weiner L M; Marks C; **Marks J D**
CS Department of Anesthesia, University of California, San Francisco 94110, USA.
SO JOURNAL OF MOLECULAR BIOLOGY, (1996 Nov 8) 263 (4) 551-67.
Journal code: J6V. ISSN: 0022-2836.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199702
EW 19970204
AB We determined the extent to which additional binding energy could be achieved by diversifying the complementarity determining regions (CDRs) located in the center of the **antibody** combining site of C6.5, a human single-chain Fv (scFv) isolated from a non-immune phage library which binds the tumor antigen c-**erbB-2**. CDR3 of the light (V(L)) and heavy (V(H)) chain variable region of C6.5 were sequentially mutated, the mutant scFv displayed on phage, and higher affinity mutants selected on antigen. Mutation of V(L) CDR3 yielded a scFv (C6ML3-9) with a 16-fold lower Kd (1.0 x 10(-9) M) than C6.5. Due to its

length of 20 amino acids, four V(H) CDR3 libraries of C6ML3-9 were constructed. The greatest increase in affinity from a single library was ninefold (Kd = 1.1 x 10(-10) M). Combination of mutations isolated from separate V(H) CDR3 libraries yielded additional ninefold decreases in Kd, resulting in a scFv with a 1230-fold increase in affinity from wild-type C6.5 (Kd = 1.3 x 10(-11) M). The increase in affinity, and its absolute value, are comparable to the largest values observed for **antibody** affinity maturation in vivo or in vitro and indicate that mutation of V(L) and V(H) CDR3 may be a particularly efficient means to increase **antibody** affinity. This result, combined with the location of amino acid conservation and substitution, suggests an overall strategy for in vitro **antibody** affinity maturation. In addition, the affinities and binding kinetics of the single-chain Fv provide reagents with potential tumor targeting abilities not previously available.

L11 ANSWER 12 OF 18 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1997:139133 BIOSIS
DN PREV199799438336
TI Engineering **antibodies** for tumor targeting using phage display.
AU Schier, Robert (1); Adams, G.; Marshall, K.; McCall, A.; Weiner, L.; Bookman, M.; **Marks, James D.**
CS (1) Dep. Anesthesia., Univ. California, San Francisco, CA USA
SO Immunotechnology (Amsterdam), (1996) Vol. 2, No. 4, pp. 290-291.
Meeting Info.: 1996 Keystone Meeting on Exploring and Exploiting Antibody and Ig Superfamily Combining Sites Taos, New Mexico, USA February 22-28, 1996
ISSN: 1380-2933.
DT Conference; Abstract
LA English

L11 ANSWER 13 OF 18 BIOSIS COPYRIGHT 1999 BIOSIS
AN 1997:139053 BIOSIS
DN PREV199799438256
TI Engineering the **antibody** combining site for high affinity binding.
AU Schier, Robert (1); McCall, Adrian; Adams, Greg; Weiner, Louis M.; **Marks, James D. (1)**
CS (1) Dep. Anesthesia, Univ. Calif., San Francisco, CA 94110 USA
SO Immunotechnology (Amsterdam), (1996) Vol. 2, No. 4, pp. 264.
Meeting Info.: 1996 Keystone Meeting on Exploring and Exploiting Antibody and Ig Superfamily Combining Sites Taos, New Mexico, USA February 22-28, 1996
ISSN: 1380-2933.
DT Conference; Abstract
LA English

L11 ANSWER 14 OF 18 MEDLINE DUPLICATE 8
AN 96194794 MEDLINE
DN 96194794
TI Identification of functional and structural amino-acid residues by parsimonious mutagenesis.
AU Schier R; Balint R F; McCall A; Apell G; Larrick J W; **Marks J D**
CS Department of Anesthesia, University of California, San Francisco General Hospital 94110, USA.
NC U01 CA 51880 (NCI)

SO GENE, (1996 Mar 9) 169 (2) 147-55.
Journal code: FOP. ISSN: 0378-1119.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-U38323; GENBANK-U38324; GENBANK-U38325; GENBANK-U38326; GENBANK-U38327; GENBANK-U38328; GENBANK-U38329; GENBANK-U38330; GENBANK-U38331; GENBANK-U38332; GENBANK-U38333; GENBANK-U38334; GENBANK-U38335; GENBANK-U38336; GENBANK-U38337; GENBANK-U38338; GENBANK-U38339; GENBANK-U38340; GENBANK-U38341; GENBANK-U38342; GENBANK-U38343; GENBANK-U38344; GENBANK-U38345; GENBANK-U38346; GENBANK-U38347
EM 199609
AB For in vitro evolution of protein function, we previously proposed using parsimonious mutagenesis (PM), a technique where mutagenic oligodeoxynucleotides (oligo) are designed to minimize coding sequence redundancy and limit the number of amino acid (aa) residues which do not retain parental structural features. For this work, PM was used to increase the affinity of C6.5, a human single-chain Fv (scFv) that binds the glycoprotein tumor antigen, c-**erbB-2**. A phage **antibody** library was created where 19 aa located in three of the heavy (H) and light (L) chain antigen-binding loops (L1, L3 and H2) were simultaneously mutated. After four rounds of selection, 50% of scFv had a lower dissociation rate constant (koff) than the parental scFv. The Kd of these scFv ranged from twofold (Kd=7.0 x 10(-9) M) to sixfold (Kd=2.4 x 10(-9) M) lower than the parental scFv (Kd=1.6 x 10(-8) M). In higher affinity scFv, substitutions occurred at 10/19 of the positions, with 21/28 substitutions occurring at only four positions, two in H2, and one each in L1 and L3. Only the wild type (wt) aa was observed at 9/19 aa. Based on a model of C6.5, seven of the nine conserved aa have a structural role in the variable domain, either in maintaining the main chain conformation of the loop, or in packing on the H-chain variable domain. Two of the conserved aa are solvent exposed, suggesting they may play a critical role in recognition. Thus, PM identified three types of aa: structural aa, functional aa which modulate affinity, and functional aa, which are critical for recognition. Since the sequence space was not completely sampled, higher affinity scFv could be produced by subjecting functional aa which modulate affinity to a higher rate of mutation. Furthermore, PM could prove useful for modifying function in other proteins that belong to structurally related families.

L11 ANSWER 15 OF 18 MEDLINE DUPLICATE 9
AN 97209882 MEDLINE
DN 97209882
TI Efficient in vitro affinity maturation of phage **antibodies** using BIAcore guided selections.
AU Schier R; **Marks J D**
CS Department of Anesthesia, University of California, San Francisco, USA.
SO HUMAN ANTIBODIES AND HYBRIDOMAS, (1996) 7 (3) 97-105.
Journal code: A6A. ISSN: 0956-960X.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199707

EW 19970705
AB Selection of higher affinity mutant phage **antibodies** has proven less than straightforward due to sequence dependent differences in phage **antibody** expression, toxicity to Escherichia coli, and difficulty in eluting the highest affinity phage. These differences lead to selection for increased levels of expression or decreased toxicity rather than for higher affinity. In this work, we demonstrate how surface plasmon resonance as employed in the BIAcore can be used to increase the efficiency of phage **antibody** selections, yielding greater increments in affinity from a single library. A mutant phage **antibody** library was created by randomizing nine amino acids located in the V(L) CDR3 of C6.5, a human scFv which binds the tumor antigen c-**erbB-2** with a Kd of 1.6 x 10-8 M. The library was subjected to five rounds of selection in solution using decreasing concentrations of biotinylated c-**erbB-2**. After each round of selection, polyclonal phage were prepared and the rate of binding to c-**erbB-2** determined in a BIAcore under mass transport limited conditions. Determination of the rate of binding permitted calculation of the concentration, and hence percent, of binding phage present. Results were used to select the antigen concentration for the next round of selection. To determine the optimal eluent, polyclonal phage was injected in a BIAcore and eluted using one of five different solutions (10 mM HCl, 50 mM HCl, 100 mM HCl, 100 mM triethylamine, 2.6 M MgCl2). Differences were observed in eluent efficacy, which was reflected in significant differences in the affinities of phage **antibodies** isolated from the library after a round of selection using the different eluents. Use of the BIAcore to determine the optimal eluent and guide the antigen concentration used for selection yielded a C6.5 mutant with a 16 fold reduction in Kd (Kd = 1.0 x 10-9 M). This represents at least a twofold greater increment in affinity than previously obtained from a single library of phage **antibodies** which bind antigens.

L11 ANSWER 16 OF 18 MEDLINE DUPLICATE 10
AN 96144830 MEDLINE
DN 96144830
TI Isolation of high-affinity monomeric human anti-c-**erbB-2** single chain Fv using affinity-driven selection.
AU Schier R; Bye J; Apell G; McCall A; Adams G P; Malmqvist M; Weiner L M; **Marks J D**
CS Department of Anesthesia and Pharmaceutical Chemistry, University of California, San Francisco 94110, USA.
NC U01 CA 51880 (NCI)
SO JOURNAL OF MOLECULAR BIOLOGY, (1996 Jan 12) 255 (1) 28-43. Journal code: J6V. ISSN: 0022-2836.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199605
AB The use of **antibodies** to target tumor antigens has had limited success, partially due to the large size of IgG molecules, difficulties in constructing smaller single chain Fv (scFv) **antibody** fragments, and immunogenicity of murine **antibodies**. These limitations can be overcome by selecting human scFv directly from non-immune or

semi-synthetic phage **antibody** libraries; however, the affinities are typically too low for therapeutic application. For hapten antigens, higher-affinity scFv can be isolated from phage **antibody** libraries where the VH and VL genes of a binding scFv are replaced with repertoires of V genes (chain shuffling). The applicability of this approach to protein binding scFv is unknown. For this work, chain shuffling was used to increase the affinity of a non-immune human scFv, which binds the glycoprotein tumor antigen c-**erbB-2** with an affinity of 1.6 x 10(-8) M. The affinity of the parental scFv was increased sixfold (Kd = 2.5 x 10(-9) M) by light-chain shuffling and fivefold (Kd = 3.1 x 10(-9) M) by heavy-chain shuffling, values comparable to those for **antibodies** against the same antigen produced by hybridomas. When selections were performed on antigen immobilized on polystyrene, spontaneously dimerizing scFv were isolated, the best of which had only a slightly lower Kd than wild type (Kd = 1.1 x 10(-8) M). These scFv dimerize on phage and are preferentially selected as a result of increased avidity. Compared to scFv which formed only monomer, dimerizing scFv had mutations located at the VH-VL interface, suggesting that VH-VL complementarity determines the extent of dimerization. Higher-affinity monomeric scFv were only obtained by selecting in solution using limiting concentrations of biotinylated antigen, followed by screening mutant scFv from bacterial periplasm by koff in a BIAcore. Using the proper selection and screening conditions, protein binding human scFv with affinities comparable to murine hybridomas can be produced without immunization.

L11 ANSWER 17 OF 18 MEDLINE DUPLICATE 11
AN 1998040666 MEDLINE
DN 98040666
TI In vitro and in vivo characterization of a human anti-c-**erbB-2** single-chain Fv isolated from a filamentous phage **antibody** library.
AU Schier R; **Marks J D**; Wolf E J; Apell G; Wong C; McCartney J E; Bookman M A; Huston J S; Houston L L; Weiner L M; et al
CS Department of Anesthesia and Pharmaceutical Chemistry, University of California, San Francisco, USA.
NC U01 CA 51880 (NCI)
SO IMMUNOTECHNOLOGY, (1995 May) 1 (1) 73-81.
Journal code: CR0. ISSN: 1380-2933.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199803
EW 19980302
AB BACKGROUND: **Antibody**-based reagents have failed to live up to their anticipated role as highly specific targeting agents for cancer therapy. Targeting with human single-chain Fv (sFv) molecules may overcome some of the limitations of murine IgG, but are difficult to produce with conventional hybridoma technology. Alternatively, phage display of **antibody** gene repertoires can be used to produce human sFv. OBJECTIVES: To isolate and characterize human single chain Fvs which bind to c-**erbB-2**, an oncogene product overexpressed by

30-50% of breast carcinomas and other adenocarcinomas. STUDY DESIGN: A non-immune human single-chain Fv phage **antibody** library was selected on human c-erbB extracellular domain and sFv characterized with respect to affinity, binding kinetics, and in vivo pharmacokinetics in tumor-bearing scid mice. RESULTS: A human single-chain Fv (C6.5) was isolated which binds specifically to c-**erbB-2**. C6.5 is entirely human in sequence, expresses at high level as native protein in E. coli, and is easily purified in high yield in two steps. C6.5 binds to immobilized c-**erbB-2** extracellular domain with a Kd of 1.6 x 10(-8) M and to c-**erbB-2** on SK-OV-3 cells with a Kd of 2.0 x 10(-8) M, an affinity that is similar to sFv produced against the same antigen from hybridomas. Biodistribution studies demonstrate 1.47% injected dose/g tumor 24 h after injection of 125I-C6.5 into scid mice bearing SK-OV-3 tumors. Tumor:normal organ ratios range from 8.9:1 for kidney to 283:1 for muscle. CONCLUSIONS: These results are the first in vivo biodistribution studies using an sFv isolated from a non-immune human repertoire and confirm the specificity of sFv produced in this manner. The use of phage display to produce C6.5 mutants with higher affinity and slower k(off) would permit rigorous evaluation of the role of **antibody** affinity and binding kinetics in tumor targeting, and could result in the production of a therapeutically useful targeting protein for radioimmunotherapy and other applications.

L11 ANSWER 18 OF 18 MEDLINE
AN 93111402 MEDLINE
DN 93111402
TI The effect of interferon gamma on epidermal growth factor receptor expression in normal and malignant ovarian epithelial cells.
AU Boente M P; Berchuck A; Rodriguez G C; Davidoff A; Whitaker R; Xu F J; **Marks J**; Clarke-Pearson D L; Bast R C Jr
CS Department of Obstetrics and Gynecology, Duke University Medical Center, Durham, NC 27710..
NC CA 39930 (NCI)
SO AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY, (1992 Dec) 167 (6) 1877-82.
Journal code: 3NI. ISSN: 0002-9378.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals; Cancer Journals
EM 199303
AB OBJECTIVE: We examined the effect of interferon gamma on proliferation and epidermal growth factor receptor expression in ovarian cancer cell lines and normal ovarian epithelial cells. STUDY DESIGN: The tritiated thymidine incorporation assay was used to assess the effect of interferon gamma on proliferation. Scatchard analysis of anti-epidermal growth factor receptor **antibody** binding, and Western blotting of immunoprecipitates was used to assess the effect of interferon gamma on epidermal growth factor receptor expression. RESULTS: Although interferon gamma elicited 30% to 40% decreases in proliferation, epidermal growth factor receptor expression was strikingly increased in all four ovarian cancer cell lines. Scatchard analysis indicated that this increase occurred primarily at the

cell surface, but total cellular receptor levels also were increased. In contrast, interferon gamma treatment of normal ovarian epithelial cells affected neither proliferation nor epidermal growth factor receptor levels. CONCLUSION: Because the up-regulation of epidermal growth factor receptors by interferon gamma appears to be confined to malignant cells, interferon gamma may facilitate immunotherapy and imaging of ovarian cancers by means of immunoconjugates directed against the epidermal growth
factor receptor.